PHYSIOLOGY IN MEDICINE

In collaboration with

The American Physiological Society, Thomas E. Andreoli, Editor

# Apoptotic Mechanisms in Acute Renal Failure

Norishi Ueda, MD, Gur P. Kaushal, PhD, Sudhir V. Shah, MD

It has been generally accepted that a catastrophic breakdown of regulated cellular homeostasis, known as necrosis, is the mode of cellular injury in various forms of acute renal failure. One of the major advances in our understanding of cell death has been the recognition that the pathways traditionally associated with apoptosis as described in the landmark study by Kerr, Wyllie, and Currie in 1972 may be very critical in the form of cell injury associated with necrosis. The pathway that is followed by the cell varies with both nature and severity of insults and may evolve from an apoptotic to a necrotic form of cell death. It is also likely that there are some common pathways that are shared and regulated in the two modes of cell death. In this review, we first describe evidence for the role of apoptotic pathways in ischemic acute renal failure, and then consider the potential mechanisms that may participate in this model of acute renal tubular injury. We then summarize the current information of apoptotic pathways related to other common causes of acute renal failure including endotoxin-induced, toxic acute renal failure and transplant rejection. A better understanding of the mechanisms of apoptosis could lead to safer and more specific therapeutic interventions for acute renal failure. Am J Med. 2000;108: 403–415. ©2000 by Excerpta Medica, Inc.

The mortality in patients with acute renal failure has remained unchanged since the mid-1960s and remains high at approximately 50%. It is unlikely that the high mortality rate of the disease will be reduced until we have a better understanding of the cellular and molecular mechanisms of renal tubular cell injury and recovery. It has long been recognized that in acute renal failure induced by ischemia, endotoxemia, nephrotoxins, or other causes, renal tubule cells die in a catastrophic breakdown of regulated cellular homeostasis, known as necrosis. In this form of cell death, cells swell and lose plasma membrane integrity, thereby releasing a variety of inflammatory mediators by rupture of the cells (1–3). The general concepts of apoptosis have been covered in other reviews in this journal. Over the last decade there is an accumulating evidence suggesting the contribution of apoptosis, as originally described in the landmark paper by Kerr et al (1) in 1972, to the pathogenesis of a wide variety of human diseases (4).

One of the major advances in our understanding of cell death has been the recognition that the pathways traditionally associated with apoptosis may be very critical in the form of cell injury associated with necrosis. Along with the rapid increase in the number of publications and interest in the study of cell death has come some confusion of the terminology. In our view, the term apoptosis should be applied only when there are morphological criteria as described in the original paper by Kerr et al (1). In apoptosis, nucleus and cytoplasm condense, and dying cells often explode into a series of membrane-bound condensed apoptotic bodies (1–3). Cytoplasmic organelles including mitochondria are largely intact, and affected cells are phagocytized by macrophages or adjutant viable cells with little leakage of cellular contents, thus evoking minimal inflammation. DNA fragmentation resulting from endonuclease activation has been considered as one of the biochemical hallmarks of apoptosis and was often equated with apoptosis because it was invariably associated with apoptosis. This also led to the earlier belief that both chromatin condensation and DNA fragmentation were a result of endonuclease activation. However, recent studies equating DNA fragmentation with apoptosis are problematic because of the following: chromatin condensation and DNA fragmentation are regulated by different metabolic pathways [cited in (3)]; apoptosis can occur in the absence of DNA fragmentation (3); and DNA fragmentation often occurs with morphologic evidence of only necrosis.

It is likely that many features of the cell-signaling process leading to the apoptotic form of cell death are shared with those associated with the necrotic form of cell death. The pathway that is followed by the cell is dependent on

From the Department of Medicine, Division of Nephrology, University of Arkansas for Medical Sciences, and Central Arkansas Veterans Healthcare System, Little Rock, Arkansas.

Supported in part by the Department of Defense, Office of the Navy (N00014–95–1–0583), the National Institutes of Health (R01 DK47990), and a VA Merit Review Award.

Requests for reprints should be addressed to Norishi Ueda, MD, Department of Medicine, Division of Nephrology, University of Arkansas for Medical Sciences, 4301 W. Markham, Slot 501, Little Rock, Arkansas 72205.



0002-9343/00/$–see front matter
PII S0002-9343(00)00311-9

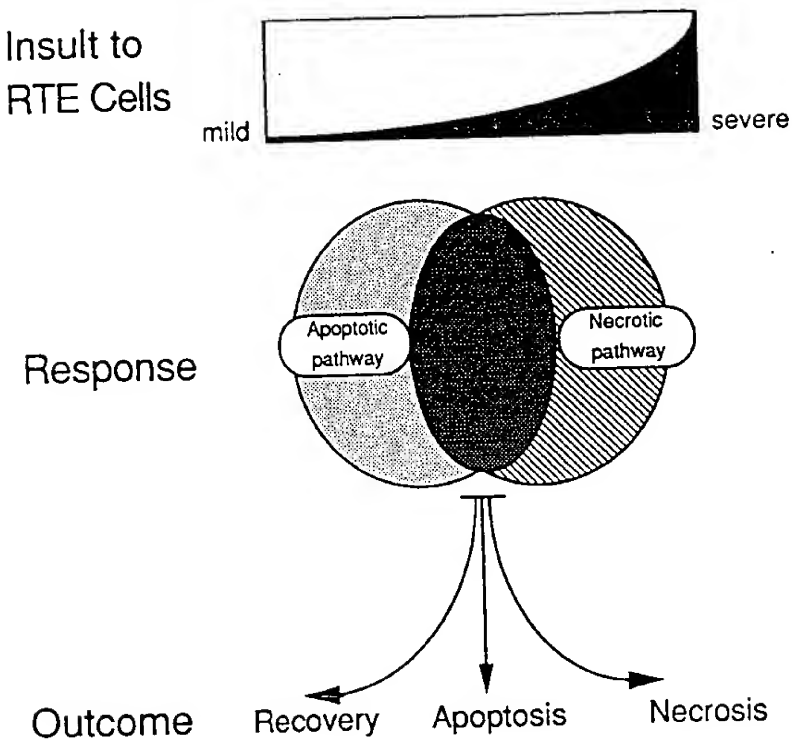


**Figure 1.** Outcome of renal tubular epithelial (RTE) cells following noxious insults. (From: Ueda N, Shah S. Role of endonucleases in renal tubular epithelial cell injury. *Experimental Nephrol.* 2000;8:8–13. Reprinted with permission of Karger.)

both the nature and severity of insults, evolving from apoptotic to necrotic cell death. Thus, it is now recognized that the same insult in a mild form can lead to apoptosis and when severe can lead to necrosis (Figure 1). As an example, the several recent studies showed that mild depletion of adenosine triphosphate (ATP) induces apoptosis and severe depletion leads to necrosis (5–7), indicating that severity of stimuli determines the cell fate. In this review, we first describe evidence for the role of apoptotic pathways in ischemic acute renal failure, and then consider the potential mechanisms that may participate in this model of acute renal tubular injury. We then summarize the current information on apoptotic pathways related to other common causes of acute renal failure including endotoxin-induced and toxic acute renal failure and transplant rejection. Other reviews on the role of apoptosis in renal tubular cell injury have also been published (3,8).

## EVIDENCE FOR APOPTOTIC MECHANISMS IN ACUTE RENAL FAILURE

### *Ischemic Acute Renal Failure*

It has long been recognized that necrosis is a major form of cell death associated with ischemic acute renal failure. However, there is accumulating evidence indicating a role of apoptotic pathways in in vitro and in vivo models of acute renal failure (Table 1). One of the first studies to document the occurrence of apoptosis in ischemic acute renal failure was a study by Schumer et al (9) in which apoptosis was observed in rat kidney cortex 12 hours after reperfusion. Since then there are a number of studies in which the DNA laddering and/or morphologic change of apoptosis have been described in ischemia/reperfusion injury to kidneys (10–14). Based on the information in the literature, the in vivo studies of ischemia/reperfusion so far demonstrate apoptosis only during the reperfusion period.

In general, the in vitro studies have shown apoptosis and DNA fragmentation in renal tubular cells during hypoxia as well as reoxygenation. Recent studies have shown DNA fragmentation in isolated perfused rat kidneys subjected to hypoxia (15), and apoptosis in renal tubule cells including LLC-PK1 cells (7), MDCK cells (6, 7), and mouse proximal tubule cells subjected to chemical hypoxia (16), as well as rat kidney proximal tubules subjected to ATP depletion (17). In our previous studies, we demonstrated that hypoxia/reoxygenation injury results in DNA fragmentation without morphologic evidence of apoptosis in isolated rat proximal tubules (18). A similar finding has been reported after in vivo ischemia/reperfusion injury in which DNA fragmentation is associated with morphologic features of necrosis rather than apoptosis (19).

Table 1. Evidence for Apoptosis Based on In Vitro and In Vivo Models of Ischemic Acute Renal Failure

| | Source | DNA Fragmentation | Apoptotic Morphology | Comments | References |
|---|---|---|---|---|---|
| In Vitro | | | | | |
| Hypoxia/reoxygenation | Rat TEC | + | + | Severe oxygen depletion causes necrosis. | 6 |
| | MDCK, LLC-PK1 | + | + | Longer ATP depletion causes necrosis. | 6,7 |
| | Mouse PTC | + | + | Maximal ATP depletion causes necrosis. | 16 |
| In Vivo | | | | | |
| Ischemia/reperfusion | Rat | + | + | Apoptosis occurs after reperfusion. | 9 |
| | Rat | + | + | Apoptosis occurs in TAL after reperfusion. Fas increases in TAL and DTC. | 12 |
| | Rat | + | ? | DNA damage occurs in TAL. | 15 |
| | Rat | + | ? | DNA damage occurs after reperfusion. | 11 |
| | Rat | + | + | Apoptosis occurs during reperfusion with increased p53 and c-myc. Calcium blockade prevents apoptosis. | 14 |
| | Rat | + | + | c-myc and c-fos increase during reperfusion. | 10 |
| | Human allograft | + | ? | DNA damage occurs in tubules of cadaveric kidney during reperfusion | 117 |

TEC = tubular epithelial cells; MDCK = canine kidney distal tubular cell line; LLC-PK1 = porcine kidney proximal tubular cell line; ATP = adenosine triphosphate; PTC = proximal tubule cell; TAL = thick ascending limbs; DTC = distal tubule cells.

## POTENTIAL MECHANISMS AND MODULATORS OF APOPTOSIS IN ISCHEMIC ACUTE RENAL FAILURE

The potential role of apoptotic pathways in a model of injury has generally been delineated based on morphologic changes of apoptosis or the presence of DNA fragmentation. These appear to be terminal events in the apoptotic pathway. In the last few years there is a large body of the literature examining the signaling pathway, which either initiates or modulates apoptosis. In the following section, we describe an overview of apoptotic mechanisms derived from observations from other model systems (Figure 2) and describe specific observations that have been reported related to acute renal failure.

### *Cell Surface Receptors*

The best characterized cell surface "death receptors" are the members of the tumor necrosis factor (TNF) superfamily, including Fas (also called CD95 or Apo 1) and TNFRI (also called p55 or CD120a) (20,21). A cell surface molecule, Fas ligand (FasL), binds to Fas receptor and TNF binds to TNFRI. This ligation leads to clustering of the receptors' death domains that recruit their adaptor proteins, which bind to the initiator procaspase-8 through the death domains forming a death-inducing signaling complex. This ligation between cell death recep-

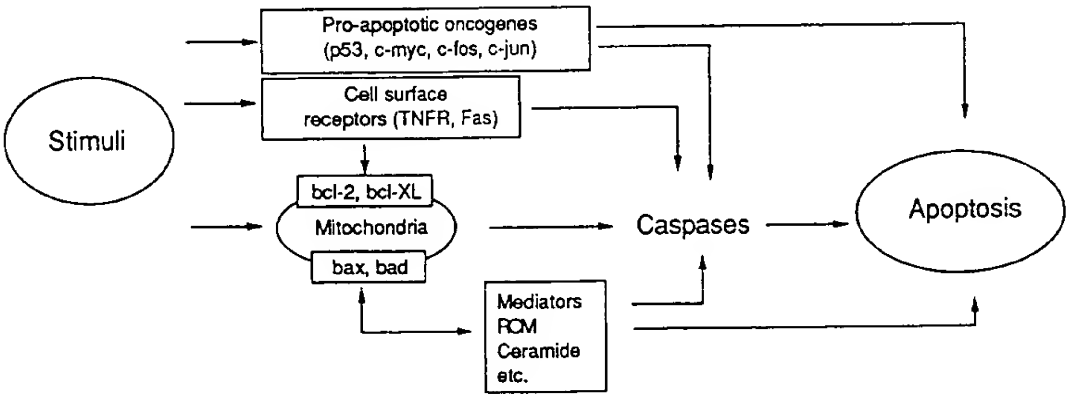


Figure 2. Simplified schema of potential mechanisms of apoptosis in acute renal tubular injury.

tor, adaptor molecules, and the initiator procaspase results in activation of caspases (see below). Renal tubule cells express Fas (22) and TNF-α (23) and addition of TNF-α to renal tubule cells has been shown to induce or enhance apoptosis (24,25), indicating that TNF-α–initiated apoptotic pathway may be involved in renal tubular injury. In fact, TNF-α level has been shown to be increased in regenerative tubules in ischemia/reperfusion injury (26). Similarly, anti-Fas antibodies have been shown to induce apoptosis in renal tubule cells (22,27). The expression of Fas and FasL has been shown to be increased in distal tubules of the outer medulla in mice ischemic kidney after reperfusion, and the number of apoptotic cells was significantly reduced in *lpr/lpr* B6 mice with low Fas level as compared with wild-type B6 mice (12). These data indicate the potential of Fas-dependent cell signaling in apoptosis associated with acute renal tubular injury. Additionally, the proapoptotic ligand Siva for CD27, another member of the TNF superfamily, has been shown to be increased in proximal tubule cells of ischemic kidneys after reperfusion (13), although its role in renal injury is not known. Taken together, these data suggest that after an insult, the binding of ligands to the TNF superfamily of cell surface receptors occurs and thereby has the potential to initiate the apoptotic process in ischemic acute renal failure.

### *Growth Factors*

Cells require growth factors for their survival, and deficiency of these factors induces apoptosis. The simplest example is that renal tubule cells in serum-free media undergo apoptosis and are rescued when epidermal growth factor (EGF) or high-dose insulin is repleted (5). Although the mechanisms of growth factors in modulating apoptosis are not precisely known, the deficiency of these factors has been shown to trigger the ligation of cell surface receptors, alteration of proapoptotic and antiapoptotic oncogenes, and other mediators of the apoptotic signaling pathway.

Alteration of expression of growth factors and their receptors has been reported in various kidney diseases. For example, the level of epidermal growth factor (EGF) has been shown to be decreased during the early phase of reperfusion following ischemia, and it returns to the basal level during the recovery phase of ischemia/reperfusion injury (28). Similar changes in the level of EGF have been reported in urine from humans with acute renal failure (29). On the other hand, hepatocyte growth factor (HGF) has been shown to be increased in ischemia/reperfusion injury (30) and in the urine of patients with acute renal failure (31). HGF has been shown to rescue apoptosis in other cells by interfering in the Fas pathway (32). Recent studies demonstrated that these growth factors accelerate the recovery of ischemic acute renal failure (33,34), indicating that alteration of growth factors plays an important role in apoptosis as well as in regeneration associated with ischemic renal tubular cell injury.

In contrast, one of the growth factors, transforming growth factor-β1 (TGF-β1), has been shown to induce apoptosis in renal tubule cells in vitro (35) and in the kidney of transgenic mice in vivo (36). The level of TGF-β1 has been demonstrated to be increased in ischemia/reperfusion (37). Although the role of this growth factor is not clear in ischemic acute renal failure, the balance between TGF-β1 that functions as a proapoptotic effector and other growth factors that protect against apoptosis may be a crucial determinant for cytotoxicity of this model of injury.

### *Proapoptotic and Antiapoptotic Oncogenes*

Within cells, there is a machinery consisting of proapoptotic oncogenes (p53, c-myc, c-fos, and some members of bcl-2, bax, or bad) that promote apoptosis (Figure 2) and antiapoptotic oncogenes (bcl-2 and bcl-$X_L$) that function as suppressors of apoptosis, and the balance between these oncogenes may be a determinant of apoptosis or cell survival. The tumor suppressor gene p53 is involved in cell cycle arrest, apoptosis, control of genome integrity, and DNA repair (38,39). Under some conditions, p53 induces transcriptional transactivation of effector genes of apoptosis including p53-responsive proapoptotic genes, bcl-2 antagonist bax, Fas, and other mediators (38–40). Activation of nuclear gene p53 in response to DNA damage results in either apoptosis or cell cycle arrest through inhibition of cyclin-dependent kinases to allow DNA repair. The nuclear proto-oncogene, c-myc, also has been implicated in the control of cell proliferation, differentiation, and apoptosis (41). It is well established that the partner protein Max is essential for c-Myc-mediated apoptosis whereas Mad or Mxi repress c-Myc function. Like c-myc, proto-oncogenes c-fos and c-jun encoding AP-1 (Fos/Jun) transcription factors have been shown to play a role in cell differentiation and apoptosis (42). The mRNA expression of these genes is increased in some cell types following growth factor withdrawal, and the inhibition of these genes rescues apoptosis, whereas in other cells in different settings, these genes exert a protective effect for apoptosis [reviewed in (42)].

There is limited information of a role of oncogenes in apoptosis associated with ischemic acute renal failure. Regarding the proapoptotic effectors, recent in vivo studies showed upregulation of p53 (10,14), c-myc (10,14), c-fos, and c-jun (43,44) in renal tubule cells of ischemic kidney during reperfusion. However, one study demonstrated that in the ex vivo perfused kidney, c-fos mRNA increased during ischemia without reperfusion (45). mRNA expression of these genes has been shown to be inhibited by *N*-acetylcysteine (44,46), suggesting a role of oxidants or the redox state in regulation of these genes (42). The proto-oncogenes, c-myc, c-fos, and c-jun are

immediate early growth response genes and also regulate mitogenic activity for cell growth and proliferation (42). The replacement of injured cells and cell proliferation are necessary for recovery. Both apoptotic and mitotic effects of these genes may facilitate removal and recovery of injured tubular epithelium in acute renal failure, although it is not known how the paradoxical effects of these genes are regulated.

The Bcl-2 family of proteins is the best characterized apoptotic effectors, and some members (Bcl-2 and Bcl-$X_L$) function as antiapoptotic factors and others (Bax, Bad or Bcl-$X_s$) promote apoptosis [reviewed in (47)]. The Bcl-2 family of proteins modulates mitochondrial cytochrome c release, alteration of mitochondrial permeability transition (MPT) that leads to free radical production (48), ATP depletion (49), and release of other proteins such as apoptosis-inducing factors and caspases (48), all of which are potential factors of inducing apoptosis. Bcl-2 or Bcl-$X_L$ prevents these phenomena, thereby suppressing apoptosis, while Bax or Bad accelerates the apoptotic process by inhibiting the protective function of Bcl-2 or Bcl-$X_L$.

Despite extensive studies in other fields, there is only limited information on a role of bcl-2 family genes in ischemic acute renal failure. In vivo Bcl-2 and Bax proteins have not been detected in the kidneys during ischemia (50,51), whereas bcl-2 mRNA expression was increased after reperfusion (51). As with the in vivo situation, the study by Saikumar et al (17) showed that in cultured rat proximal tubule cells, Bcl-2 protein could not be detected. Overexpression of bcl-2 can prevent release of mitochondrial cytochrome c thereby suppressing apoptosis in renal tubule cells induced by hypoxia/reoxygenation (17). Overexpression of bcl-2 also rescues necrosis induced by hypoxia/reoxygenation (17) or oxidants in renal tubular cells by preserving mitochondrial integrity and increasing the mitochondrial capacity of buffering calcium (52). These data imply that the Bcl-2 family of proteins can regulate both apoptotic and necrotic pathway, and that these two forms of cell death may share some common signaling pathway.

The expression of bcl-2 mRNA and Bcl-2 protein has been demonstrated in the regenerating proximal tubules of the outer medulla, indicating that the expression of antiapoptotic proto-oncogenes correlates well with the regenerating process of renal tubules (51). On the other hand, the expression of bax mRNA and Bax protein has been shown to be increased 1 to 7 days following ischemia, and was localized in the distal tubules, cortical collecting duct and medullary thick ascending limb of Henle's loop but not the proximal tubules (51). These data imply that the regulation of antiapoptotic and proapoptotic oncogenes may vary with cell types in the kidney and that this may account for the occurrence of apoptosis and susceptibility to apoptotic stimuli during the acute phase of acute renal failure as well as the regeneration process during the recovery phase of the disease.

### *Mitochondria*

It is now widely accepted that mitochondria play a key role in the decision of cells to undergo apoptosis or necrosis (49) and in regulating the apoptotic process (48, 49). Evidence for a role of mitochondria in apoptosis comes from the following: 1) The change in mitochondrial permeability transition (MPT) precedes and triggers nuclear apoptosis whereas the stabilization of MPT by antiapoptotic proteins (Bcl-2 or Bcl-$X_L$) or cyclosporine A rescues apoptosis. 2) The volume dysregulation of mitochondria when PT pore opening occurs can result in the release of cytochrome c, which in turn activates caspase-9 by forming a complex with Apaf-1, procaspase-9, and dATP (53). Mitochondria dysfunction may also release other proteases such as caspases and apoptosis-inducing factors (48) under some circumstances. 3) The change in MPT results in production of reactive oxygen metabolites (ROM) and cessation of mitochondrial ATP production leading to ATP depletion. The change in MPT also leads to the increase in intracellular calcium, known as an inducer of apoptosis (54), and this in turn results in further dysfunction of mitochondria (49). The redox state in mitochondria is also a determinant for apoptosis (54). When MPT results in severe ATP depletion, necrotic cell death ensues, but when MPT occurs without ATP depletion, apoptosis occurs (49). This may be relevant to the fact that mild ATP depletion induces apoptosis whereas severe depletion of ATP induces necrosis in renal tubule cells (5–7). As described earlier, an in vitro study showed that ATP depletion results in release of mitochondrial cytochrome c and translocation of Bax protein from cytosolic to mitochondrial membrane and that overexpression of Bcl-2 prevented mitochondrial cytochrome c release and apoptosis in renal tubule cells (17).

### *Reactive Oxygen Metabolites*

DNA fragmentation and/or morphological evidence of apoptosis has been reported in several in vitro and in vivo models of acute renal failure in which reactive oxygen metabolites (ROM) have been implicated [reviewed in (55)], including hypoxia/reoxygenation and ischemia/reperfusion injury, and HgCl2- and cisplatin-induced injury. The role of ROM (56,57) in apoptosis has been supported by the following: 1) ROM can induce apoptosis in a variety of cells, including renal tubule cells (58,59); 2) the generation of these metabolites and depletion of intracellular glutathione level precede and are associated with apoptosis; 3) oxidized lipoprotein and lipid hydroperoxides can induce apoptosis; 4) apoptosis can be blocked by inhibiting or neutralizing ROM, including renal tubule cells following ischemia/reperfusion injury (44); and 5) down-regulation of superoxide dismutase

causes apoptosis. The extent of ROM formation and the cell's ability to detoxify them may be a determinant for apoptosis or necrosis, since mild stimuli cause apoptosis and severe insults induce necrosis in models of oxidant injury (16,58,60).

ROM are generated in response to a wide variety of apoptotic stimuli. Intracellular sources of ROM include mitochondria, which appear to be the most important source, the microsomal cytochrome P450 system, and plasma membrane NAD(P)H oxidases (56). Oxidants trigger a variety of mediators involved in apoptosis including growth factor-β1 (61), Apo-1/Fas ligand (62), mitochondrial permeability transition (49), p53 (63), caspases (64), ceramide (65,66), and others (67–69). In addition, oxidant stress also results in reduction of redox state. The function of many proteins, including growth factor receptors, protein kinases such as mitogen-activated protein (MAP) kinases, protein phosphatases, G proteins, transcription factors, AP-1, NF-kB, and p53, are regulated by their redox state [reviewed in (57,68)]. AP-1, characterized as an antioxidant-responsive factor, may also be a modulator of apoptosis (68). Thus, cellular redox equilibrium may prevent apoptosis by suppressing "death" genes through antioxidant-responsive transcription factors.

Little is known of cellular targets for ROM in acute renal tubular injury. As described, the potential relevance of ROM in regulation of oncogenes may be the alteration of bcl-2 expression in renal tubule cells in several models of acute renal failure in which oxidants are implicated, including ischemic acute renal failure (51) as well as the protective effect of antioxidants on expression of pro-apoptotic genes in acute renal failure (44,46). As apoptotic effectors, activation of Jun N-terminal kinase (JNK)/MAP kinases has been shown to inhibit cell growth and promote apoptosis (70). Recent studies showed that these kinases are downstream of ROM (67). In fact, activation of JNK/MAP kinases has been demonstrated in the heart and kidney following ischemia/reperfusion injury (11). However, it remains to be elucidated whether these mediators regulated by ROM play a role in apoptosis in acute renal tubular injury.

## *Caspases*

The caspases (ICE/CED3 family of proteases) are a family of cell death proteases that play a key role in the execution of apoptosis. The term caspases signifies two distinct properties of these enzymes, in which "c" refers to the cysteine proteases and aspase denotes their specificity to cleave after aspartic acid (71). The first caspase was discovered in 1993 when it was shown that cell death gene CED3 in *Caenorhabditis elegans* has sequence homology to caspase-1, which was then called the interleukin-1β converting enzyme (72). So far, 14 members of the caspase family have been identified from mammalian cells (73,74). Overexpression of executioner and initiator caspases in transfected cells results in DNA fragmentation and cell death in a variety of transfected mammalian cell lines (75,76). At least 40 different protein substrates for caspases have been recognized, including DNA repair enzymes (76), DNA fragmentation factor, which is responsible for internucleosomal DNA cleavage (77,78), nuclear structural proteins, cytoskeleton proteins, and caspases themselves (73,75). It is assumed that these or more unknown substrates cleaved by caspases are responsible for the changes that occur during the process of apoptosis.

Currently there is limited information on the role of caspases in ischemic renal tubular cell injury. In our previous studies, we have demonstrated the participation of caspases in hypoxic injury to renal tubular cells (79) and ischemia/reperfusion injury to kidneys (80). We have shown that chemical hypoxia with antimycin A results in increased caspase activity that precedes DNA damage and cell death and that the inhibition of caspases prevents hypoxia-induced DNA damage and cell death in LLC-PK1 cells (79). Partial ATP depletion in MDCK cells induced by chemical hypoxia also has been shown to result in apoptosis with a marked increase in the activation of caspase-8 (81). In a related study, activation of caspase-3 during hypoxia or ATP depletion has been shown to be accompanied by cytochrome c release from mitochondria (17). Using an in vivo model of ischemia/reperfusion injury, we have demonstrated both activation as well as increased expression of caspase-1 and caspase-3 mRNA (80). These data indicate the differential regulation of caspases and their role for apoptosis in ischemic acute renal failure.

## *Ceramide*

It is now recognized that the products formed by hydrolysis of membrane phospholipids can serve as key molecules in cell signaling pathways (82,83). Recently, ceramide, a metabolite of sphingolipids, has been implicated as playing an important role in the cell signaling pathway involved in growth arrest, differentiation, and apoptosis (83). Exogenous ceramide or other analogs can induce apoptosis in a variety of cells, and an increased ceramide level has been demonstrated in apoptosis in response to stimuli (82,83).

Ceramide is generated by two major pathways: condensation of sphinganine or sphingosine and fatty acyl-CoA by ceramide synthase, or degradation of sphingomyelin mediated by sphingomyelinases (82,83). Most studies have shown that increased ceramide generation is mediated by sphingomyelinases (82,83). In the kidney, recent studies by Zager et al (84,85) showed an increased ceramide level during reperfusion of ischemic mouse kidney and in hypoxic renal tubule cells accompanied by a decrease in sphingomyelinase activity. In our previous

studies, we have shown that subjecting renal tubule cells to hypoxia results in a rapid increase in ceramide generation due to ceramide synthase activation and that inhibition of ceramide synthase prevents hypoxia-induced ceramide generation, DNA fragmentation, and necrotic cell death (86,87). Exogenous ceramide results in DNA damage and cell death similar to the effect of hypoxia. These data suggest that ceramide may be a regulator of renal tubular cell injury. Ceramide can trigger apoptosis through a wide variety of intracellular targets: ceramide-activated protein kinase (CAPK), JNK, or MAP kinases, proto-oncogenes such as c-myc, Vav (Ras-activating guanine nucleotide exchange factor), ceramide-activated protein phosphatase (CAPP), Raf-1 kinase, phospholipases, PKC ζ, transcription factors such as NF-kB, and caspase-3 [reviewed in (83)]. The potential relevance of ceramide to renal tubular injury has also been suggested by the fact that TNF-α, a well-known inducer of ceramide, induces or enhances apoptosis in renal tubule cells (88) and the increase in TNF can be seen in several models of acute renal failure. Activation of JNK/MAP kinases (11) and caspases occurs in ischemia/reperfusion injury to kidneys or hypoxia to renal tubular cells (79,80), and ceramide is capable of regulating these mediators. Thus, it is possible that ceramide may be a secondary effector of both apoptosis and necrosis in acute renal tubular injury. Further studies would be necessary to determine the role of ceramide in apoptosis in renal tubular injury.

## APOPTOTIC PATHWAYS IN ENDOTOXIN-INDUCED, TOXIC, AND OTHER CAUSES OF ACUTE RENAL FAILURE

### *Endotoxin-Induced Acute Renal Failure*

Endotoxemia is one of the important causes of acute renal failure, and apoptosis has been shown to be associated with this model of renal injury (Table 2). Recent in vitro studies showed that apoptosis occurs in renal tubule cells in response to verotoxins (25) and *Escherichia coli* Shiga-like toxins (89,90). These experimental data have been supported by clinical studies in which apoptosis was seen in renal tubule cells from patients with hemolytic uremic syndrome (25,90). The mechanisms of endotoxin-induced apoptosis in renal tubular cell injury are not known; however, there are several lines of evidence for a role of cell surface receptor-initiated cell signaling, anti-apoptotic oncogenes, caspases, and some mediators. For example, lipopolysaccharides (LPS) and TNF-α enhance mRNA expression of Fas in renal tubule cells, and apoptosis occurs following treatment with anti-Fas antibody in the presence of LPS (27). In addition, it was shown that TNF-α enhances endotoxin-induced apoptosis in renal tubular cells (25,89). Similar observations have been obtained from in vivo studies in which LPS could induce apoptosis in the kidney (27,91) accompanied by increased expression of Fas and TNF-α (27). Additionally, the ability of plasma from patients with thrombotic thrombocytopenic purpura and sporadic hemolytic uremic syndrome to induce enhanced Fas expression and apoptosis has been demonstrated in endothelial cells (92), and this phenomenon could be inhibited by overexpression of bcl-2 and inhibitors of caspases (93). These data imply that following exposure to endotoxins, the ligation of TNF superfamily receptors occurs, thereby activating caspases leading to apoptosis, and that Bcl-2 family proteins function as a regulatory effector in this process of endotoxin-induced acute renal failure. LPS can activate NF-kB in renal tubular cells (94), which may function as both proapoptotic and antiapoptotic effectors in different cells and different settings (95,96). These data suggest that endotoxin-induced NF-kB-dependent cell signaling may also participate in the apoptotic process of endotoxin-induced acute renal failure.

### *Toxic Acute Renal Failure*

Agents used for diagnosis and treatment can trigger apoptosis in renal tubule cells (Table 2). They include chemotherapeutic agents such as cisplatin (5,97–100) and doxorubicin (101), cyclosporine A (102), radiocontrast (15,103), and thiazide diuretics (104). Antibiotics also may have the potential to induce apoptosis. An experimental study showed that gentamicin induced apoptosis in renal distal tubules during the acute phase and in proximal tubules during the recovery phase (105). Apoptosis has been shown in renal distal tubules in a patient who received an overdose of ciprofloxacin (106). Other nephrotoxins such as mercury chloride (HgCl2) (60) and cadmium (107,108) can induce apoptosis (Table 2). The severity of insults is a determinant for whether cells undergo apoptosis or necrosis, because the lower dose of agents such as cisplatin (109) and HgCl2 (60) can induce apoptosis whereas the higher dose causes necrosis.

Although the mechanisms of apoptosis in toxic acute renal failure are not known, growth factors have been implicated as modulators of apoptosis in toxic acute renal failure. An increased level of HGF has been shown in acute renal failure induced by HgCl2 (30), and a decreased level of EGF has been demonstrated in cisplatin-induced acute renal failure (30). In vitro and in vivo studies have shown the beneficial effect of administration of HGF on preventing apoptosis in renal tubule cells induced by cisplatin and HgCl2 (99,110).

Although only limited information is available, a role for oncogenes as apoptotic effectors has been suggested. Nephrotoxins can increase proapoptotic oncogenes, thereby initiating apoptosis. For example, exposure of renal tubule cells to cadmium (108) and S-(1,2-dichlorovinyl)-L-cysteine (111) has been shown to increase c-myc

Table 2. Evidence for Apoptosis from In Vitro and In Vivo Models of Endotoxin-induced and Toxic Acute Renal Failure

| | Source | DNA Fragmentation | Apoptotic Morphology | Comments | References |
|---|---|---|---|---|---|
| In Vitro | | | | | |
| Endotoxins | Human TEC | + | ? | *E. coli* Shiga toxins causes DNA damage. | 89 |
| | Human TEC | + | ? | Verotoxins cause DNA damage. CHX or TNF enhances apoptosis. | 25 |
| | Human TEC | + | + | *E. coli* Shiga-like toxin induces apoptosis. | 90 |
| Oxidant stress | LLC-PK1 | + | + | High dose of H2O2 causes necrosis. | 58 |
| | NRK | + | + | EGF prevents apoptosis. | 127 |
| Cisplatin | Mouse PTC, CDC | + | + | c-fos increases. ActD or CHX prevents apoptosis. | 98 |
| | Mouse PTC | + | + | Bcl-2 and crm A inhibit apoptosis. | 97 |
| | | + | + | High dose of cisplatin causes necrosis. | 5 |
| | Mouse CDC | + | + | HGF prevents apoptosis. | 99 |
| $HgCl_2$ | LLC-PK1 | + | + | High dose of HgCl2 causes necrosis. | 60 |
| Radiocontrast | MDCK | + | + | CHX enhances apoptosis. | 103 |
| Cadmium | LLC-PK1 | + | ? | c-myc increases but its inhibition does not affect cell death. | 108 |
| In Vivo | | | | | |
| Endotoxins | Human | + | + | Apoptosis occurs in tubule cells. | 90 |
| | Mouse | + | + | *E. coli* O157:H7 results in apoptosis in tubules. | 90 |
| Oxidants | Mouse | + | + | Apoptosis occurs in PTC. | 59 |
| Cisplatin | Rat | + | ? | DNA damage occurs in DTC and CDC. Inhibition of p21WAF1 worsens injury. | 100 |
| Radiocontrast | Rat | + | ? | DNA damage occurs in TAL. | 15 |
| Gentamicin | Rat | ? | + | Apotosis occurs in DTC during acute phase and in PTC during regenerative phase. | 105 |
| Doxorubicin | Rat | + | + | Apoptosis occurs in PTC and DTC. | 101 |
| Cyclosporine | Rat | + | + | Apoptosis in tubule and interstitial cells. Inhibition of AgII receptor and nitric oxide prevents apoptosis. | 102 |
| Thiazide | Rat | + | + | Apoptosis occurs in DTC. | 104 |
| Cadmium | Rat | + | + | Apoptosis occurs in PTC. | 107 |
| Ciprofloxacin | Human | + | + | Apoptosis occurs in TAL. | 106 |

PTC = proximal tubule cell; DTC = distal tubule cells; CDC = collecting duct cells; TEC = tubular epithelial cells; TAL = thick ascending limbs; CHX = cycloheximide; ActD = actinomycin D; TNF = tumor necrosis factor; EGF = epidermal growth factor; HGF = hepatocyte growth factor; TGF-$\beta$ = transforming growth factor-$\beta$; Ag = angiotensin.

mRNA expression. Forced c-myc expression can induce apoptosis in LLC-PK1 cells. Under the condition in which necrosis is the predominant type of cell death induced by S-(1,2-dichlorovinyl)-L-cysteine, overexpression of c-myc biases the cell death pathway toward apoptosis (111). In the same renal tubule cell line, cadmium increased c-myc mRNA expression, whereas the inhibition of c-Myc expression failed to rescue apoptosis (108). These data suggest a role of the proapoptotic oncogene, c-myc, in promoting apoptosis; however, the c-myc-dependent cell signaling pathway toward apoptosis may vary with toxins. As with other proapoptotic oncogenes, upregulation of c-fos mRNA has been shown in renal tubule cells exposed to cisplatin (97) or cadmium (46). Overexpression of bcl-2 has been shown to rescue cisplatin-induced apoptosis (97,98). In addition, it was shown that cisplatin induced more profound apoptosis in the kidney of p21-knockout mice as compared with wild type mice, indicating that a cell-cycle inhibitory protein p21, which is partly regulated by p53, may be cytoprotective by preventing DNA-damaged cells from entering the cell-cycle to die (100). These data suggest a role of proapoptotic and antiapoptotic effectors in modulating apoptosis in toxic acute renal tubular injury. However, these gene-initiated cell signaling pathways may vary with stimuli, and the severity of stimuli may determine whether cells undergo apoptosis or necrosis.

Caspases are involved in apoptosis associated with toxic acute renal failure. Activation of caspase-3 was recently shown to be accompanied by cisplatin-induced cell death in mouse proximal tubular cells (112) and in dichlorovinylcysteine (DCVC)-induced apoptosis in rat re-

Table 3. Evidence for Apoptosis In Vivo Models of Other Causes of Acute Renal Failure

| | Source | DNA Fragmentation | Apoptotic Morphology | Comments | References |
|---|---|---|---|---|---|
| In Vivo | | | | | |
| Allografts | Human | + | + | Apoptosis occurs in PTC and DTC. | 114 |
| | Human | + | + | p53 and Bax increase with rejection. | 115 |
| | Human | + | ? | Apoptosis occurs in PTC and DTC. | 116,120 |
| | Human | + | + | Fas increases. | 119 |
| Hydronephrosis due to ureteral obstruction | Rat | + | ? | EGF protects apoptosis. | 123 |
| | Rat | + | + | Apoptosis occurs in DTC and interstitium. | 125 |
| | Rat | + | + | Increased p53 and AgII receptor blockade prevents apoptosis. | 122,126 |
| | Rat | + | ? | TGF-β1 increases and EGF protects apoptosis. | 124 |
| Renal artery stenosis | Rat | + | + | Apoptosis occurs in atrophic tubules. | 128 |

Abbreviations listed in Tables 1 and 2.

nal tubular cells (113). Overexpression of crmA, a cowpox virus gene known to inhibit caspases, provided marked protection against cisplatin-induced apoptosis in mouse proximal tubular cells (97,98). In DCVC-induced apoptosis, the caspase-3 activation was associated with cleavage of focal adhesion kinase and loss of focal contacts in rat renal tubular cells. Inhibition of caspase activity with zVAD-fmk has been shown to rescue cleavage of focal adhesion kinase and apoptosis in these cells (113). Taken together these data imply that caspases function as an executioner of apoptosis induced by toxic acute renal failure.

### *Transplant Rejection and Other Causes of Acute Renal Failure*

Apoptosis has been shown in allograft kidneys from patients with acute or chronic rejection (114–116) (Table 3). It was also shown that DNA fragmentation in situ occurs during reperfusion in human renal allografts from cadaveric donors but not living-related donors (117). Although the role and mechanisms of apoptosis during rejection of allografted kidneys are not known, it appears likely that some apoptotic effectors regulate the rejection process. One of the potential effectors is a member of the TNF superfamily of cell surface receptors. Increased mRNA expression of Fas or FasL has been demonstrated in experimental models of acute and chronic rejection of allograft kidneys in mice (118) and in patients with acute rejection (119). Renal tubule cells express Fas (22) and TNF-α (23), and infiltration of cytotoxic T lymphocytes, in which Fas-FasL and TNF-α are highly expressed (20, 21), are often seen in rejected kidney allografts (120). Such lymphocytes might trigger TNF-α and Fas-FasL-mediated pathways of targeted renal tubule cells to undergo apoptosis, leading to graft rejection. Proapoptotic and antiapoptotic oncogenes may have the potential of regulating apoptosis and graft function. Increased expression of p53 and Bax proteins (115) and decreased Bcl-2 protein expression (115,121) have been shown in renal tubules of allograft kidneys with acute or chronic rejection as compared with normal functioning allografts. These data might imply that the balance between proapoptotic and antiapoptotic effectors is crucial in the regulation of apoptotic pathways for rejection and graft survival.

Apoptosis occurs in other causes of acute renal failure (Table 3). Hydronephrosis due to ureteral obstruction also has been shown to induce apoptosis or DNA fragmentation in the kidney (122–125). Although the mechanisms of apoptosis in renal injury due to ureteral obstruction are not known, increased levels of proapoptotic effectors such as TGF-β1 (124) and p53 (126) have been reported. Treatment with angiotensin-converting enzyme inhibitors has been shown to suppress p53 expression and apoptosis (126). These data indicate the potential role of ligation of angiotensin II to its type 2 receptor (AT2) in regulating apoptosis, thereby affecting remodeling or repair of renal tubule cells. Growth factors also accelerate the recovery of acute renal failure due to obstructive nephropathy (37,110,123,124) and thus play an important role in both apoptosis and mitosis in this model of renal tubular cell injury.

## THERAPEUTIC IMPLICATIONS OF APOPTOSIS

It is still premature to seek therapeutic benefits based on our current knowledge of apoptosis in human diseases, including acute renal failure. Nonetheless, treatments that can increase the apoptotic thresholds of cells may be beneficial in the diseases associated with cell loss (4). Potential treatments for acute renal failure could be pharmacologic inhibitors of apoptosis or promoters of cell survival, such as the use of growth factors to promote cell recovery after insults to kidney (110) or treatment with

antioxidants to prevent apoptosis in acute ischemic renal failure (44).

Theoretically, the modification of the genes involved in apoptosis, such as bcl-2 and caspases, could be potential treatments. However, the expression of such genes is different in every disease state or organ, and it is unlikely that most diseases are characterized by a generalized increase in either susceptibility or resistance to apoptosis (4). Additionally, one has to keep in mind that treatments that promote cell survival by modulating these genes may enhance tumor progression or development of autoimmune disease. Thus, systemic modification of such mediators of apoptosis might be problematic. Instead, one future application of current knowledge regarding apoptosis may be in designing safer and individual organ-targeted gene therapy.

## CONCLUSION

Along with a rapid growth of interest of cell biology has come new thinking regarding the apoptotic mode of cell death. In the body, individual cells or organs have evolutionary conserved pathways that regulate cell growth, differentiation, and apoptosis; and the balance among these events appears to be crucial in the maintenance and repair of organs. The cell signaling pathway toward apoptosis can be triggered and regulated differently from cell to cell or organ to organ in different settings. Additionally, it is likely that many features of the cell signaling process leading to apoptosis are shared with those associated with necrosis. The cell death pathway that is followed by the cell, namely, necrosis or apoptosis, may be dependent on both the nature and the severity of insults. These observations can be applicable to acute renal tubular injury. A better understanding of the mechanisms of apoptosis may provide safer and more specific therapeutic interventions for acute renal failure.

## REFERENCES


1. Kerr JFR, Wyllie AH, Currie AR. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br J Cancer.* 1972;26:239–257.
2. Wyllie AH. Apoptosis: an overview. *Br Med Bull.* 1997;53:451–465.
3. Ueda N, Kaushal GP, Shah SV. Recent advances in understanding mechanisms of renal tubular injury. *Adv Renal Replace Therapy.* 1997;4:1–8.
4. Thompson CB. Apoptosis in the pathogenesis and treatment of disease. *Science.* 1995;267:1456–1462.
5. Lieberthal W, Triaca V, Koh JS, et al. Role of superoxide in apoptosis induced by growth factor withdrawal. *Am J Physiol.* 1998; 275:F691–F702.
6. Allen J, Winterford C, Axelsen RA, Gobe GC. Effects of hypoxia on morphological and biochemical characteristics of renal epithelial cell and tubule cultures. *Renal Fail.* 1992;14:453–460.
7. Wiegele G, Brandis M, Zimmerhackl LB. Apoptosis and necrosis during ischaemia in renal tubular cells (LLC-PK1 and MDCK). *Nephrol Dial Transplant.* 1998;13:1158–1167.
8. Lieberthal W, Koh JS, Levine JS. Necrosis and apoptosis in acute renal failure. *Sem Nephrol.* 1998;18:505–518.
9. Schumer M, Colombel MC, Sawczuk IS, et al. Morphologic, biochemical, and molecular evidence of apoptosis during the reperfusion phase after brief periods of renal ischemia. *Am J Pathol.* 1992;140:831–838.
10. Nakajima T, Miyaji T, Kato A, et al. Uninephrectomy reduces apoptotic cell death and enhances renal tubular cell regeneration in ischemic ARF in rats. *Am J Physiol.* 1996;271:F846–F853.
11. Yin T, Sandhu G, Wolfgang CD, et al. Tissue-specific pattern of stress kinase activation in ischemic/reperfused heart and kidney. *J Biol Chem.* 1997;272:19943–19950.
12. Nogae S, Miyazaki M, Kobayashi N, et al. Induction of apoptosis in ischemia-reperfusion model of mouse kidney: possible involvement of Fas. *J Am Soc Nephrol.* 1998;9:620–631.
13. Padanilam BJ, Lewington AJP, Hammerman MR. Expression of CD27 and ischemia/reperfusion-induced expression of its ligand Siva in rat kidneys. *Kidney Int.* 1998;54:1967–1975.
14. Raafat AM, Murray MT, McGuire T, et al. Calcium blockade reduces renal apoptosis during ischemia reperfusion. *Shock.* 1997;8: 186–192.
15. Beeri R, Symon Z, Brezis M, et al. Rapid DNA fragmentation from hypoxia along the thick ascending limb of rat kidneys. *Kidney Int.* 1995;47:1806–1810.
16. Lieberthal W, Menza SA, Levine JS. Graded ATP depletion can cause necrosis or apoptosis of cultured mouse proximal tubular cells. *Am J Physiol.* 1998;274:F315–F327.
17. Saikumar P, Dong Z, Patel Y, et al. Role of hypoxia-induced Bax translocation and cytochrome C release in reoxygenation injury. *Oncogene.* 1998;17:3401–3415.
18. Ueda N, Walker PD, Hsu S-M, Shah SV. Activation of a 15-kDa endonuclease in hypoxia/reoxygenation injury without morphologic features of apoptosis. *Proc Natl Acad Sci USA.* 1995;92:7202–7206.
19. Iwata M, Myerson D, Torok-Storb B, Zager RA. An evaluation of renal tubular DNA laddering in response to oxygen deprivation and oxidant injury. *J Am Soc Nephrol.* 1994;5:1307–1313.
20. Nagata S, Golstein P. The Fas death factor. *Science.* 1995;267: 1449–1456.
21. Baker SJ, Reddy EP. Modulation of life and death by the TNF receptor superfamily. *Oncogene.* 1998;17:3261–3270.
22. Boonstra JG, Van der Woude FJ, Wever PC, et al. Expression and function of Fas (CD95) on human renal tubular epithelial cells. *J Am Soc Nephrol.* 1997;8:1517–1524.
23. Jevnikar AM, Brennan DC, Singer GG, et al. Stimulated kidney tubular epithelial cells express membrane associated and secreted TNFα. *Kidney Int.* 1991;40:203–211.
24. Ostad M, Shu WP, Kong L, Liu BCS. Ha-ras oncogene expression abrogates a pH dependent endonuclease activity of apoptosis in normal rat kidney cells. *Cancer Lett.* 1996;98:175–182.
25. Taguchi T, Uchida H, Kiyokawa N, et al. Verotoxins induce apoptosis in human renal tubular epithelium derived cells. *Kidney Int.* 1998;53:1681–1688.
26. Goes N, Urmson J, Vincent D, et al. Effect of recombinant human insulin-like growth factor-1 on the inflammatory response to acute renal injury. *J Am Soc Nephrol.* 1996;7:710–720.
27. Ortiz-Arduan A, Danoff TM, Kalluri R, et al. Regulation of Fas and Fas ligand expression in cultured murine renal cells and in the kidney during endotoxemia. *Am J Physiol.* 1996;271:F1193–F1201.
28. Schaudies RP, Nonclercq DLN, Toubeau G, et al. Endogenous EGF as a potential renotrophic factor in ischemia-induced acute renal failure. *Am J Physiol.* 1993;265:F425–434.

29. Taira T, Yoshimura A, Iizuka K, et al. Urinary epidermal growth factor levels in patients with acute renal failure. *Am J Kid Dis.* 1993;22:656–661.
30. Hammerman MR. Growth factors and apoptosis in acute renal injury. *Curr Opin Nephrol Hypertens.* 1998;7:419–424.
31. Taman M, Liu Y, Tolbert E, Dworkin LD. Increased urinary hepatocyte growth factor excretion in human acute renal failure. *Clin Nephrol.* 1997;48:241–245.
32. Kopp JB. Hepatocyte growth factor: mesenchyman signal for epithelial homeostasis. *Kidney Int.* 1998;54:1392–1393.
33. Humes HD, Cieslinski DA, Coimbra TM, et al. Epidermal growth factor enhances renal tubule cell regeneration and repair and accelerates the recovery of renal function in postischemic acute renal failure. *J Clin Invest.* 1989;84:1757–1761.
34. Miller SB, Martin DR, Kissane J, Hammerman MR. Hepatocyte growth factor accelerates recovery from acute ischemic renal injury in rats. *Am J Physiol.* 1994;266:F129–F134.
35. Nowak G, Schnellmann RG. Autocrine production and TGF-$\beta$1-mediated effects on metabolism and viability in renal cells. *Am J Physiol.* 1996;271:F689–F697.
36. Sanderson N, Factor V, Nagy P, et al. Hepatic expression of mature transforming growth factor $\beta$1 in transgenic mice results in multiple tissue lesions. *Proc Natl Acad Sci USA.* 1995;92:2572–2576.
37. Hirschberg R, Ding H. Growth factors and acute renal failure. *Sem Nephrol.* 1998;18:191–207.
38. Agarwal ML, Taylor WR, Chernov MV, et al. The p53 network. *J Biol Chem.* 1998;273:1–4.
39. Levine AJ. P53, the cellular gatekeeper for growth, and division. *Cell.* 1997;88:323–331.
40. Amundson SA, Myers TG, Fornace AJ Jr. Roles for p53 in growth arrest and apoptosis: putting on the brakes after genotoxic stress. *Oncogene.* 1998;17:3287–3299.
41. Hoffman B, Liebermann DA. The proto-oncogene c-myc and apoptosis. *Oncogene.* 1998;17:3351–3357.
42. Liebermann DA, Hoffman B. MyD genes in negative growth control. *Oncogene.* 1998;17:3319–3329.
43. Witzgall R, Brown D, Schwarz C, Bonventre JV. Localization of proliferating cell nuclear antigen, vimentin, c-Fox, and clusterin in the postischemic kidney. *J Clin Invest.* 1994;93:2175–2188.
44. DiMari J, Megyesi J, Udvarhelyi N, et al. N-acetyl cysteine ameliorates ischemic renal failure. *Am J Physiol.* 1997;272:F292–F298.
45. Joannidis M, Cantley LG, Spokes K, et al. Modulation of c-fos and egr-1 expression in the isolated perfused kidney by agents that alter tubular work. *Kidney Int.* 1997;52:130–139.
46. Wispriyono B, Matsuoka M, Igis H, Matsuo K. Protection from cadmium cytotoxicity by N-acetylcysteine in LLC-PK1 cells. *J Pharmacol Exp Ther.* 1998;287:344–351.
47. Reed JC. Bcl-2 family proteins. *Oncogene.* 1998;17:3225–3236.
48. Kroemer G, Dallaporta B, Resche-Rigon M. The mitochondrial death/life regulator in apoptosis and necrosis. *Ann Rev Physiol.* 1998;60:619–642.
49. Lemasters JJ, Nieminen AL, Qian T, et al. The mitochondrial permeability transition in cell death: a common mechanism in necrosis, apoptosis and autophagy. *Biochim Biophys Acta.* 1998;1366:177–196.
50. Eschwege P, Paradis V, Conti M, et al. Bcl-2 and Bax expression on rat ischemic kidney. *Transplant Proc.* 1998;30:2861–2862.
51. Basile DP, Liapis H, Hammerman MR. Expression of bcl-2 and bax in regenerating rat renal tubules following ischemic injury. *Am J Physiol.* 1997;272:F640–F647.
52. Ichimiya M, Chang SH, Liu H, et al. Effect of Bcl-2 on oxidant-induced cell death and intracellular Ca2+ mobilization. *Am J Physiol.* 1998;275:C832–C839.
53. Li P, Nijhawan D, Budihardjo I, et al. Cytochrome C and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. *Cell.* 1997;91:479–489.
54. Macho A, Hirch T, Marzo I, et al. Glutathione depletion is an early and calcium elevation is a late event of thymocyte apoptosis. *J Immunol.* 1997;158:4612–4619.
55. Baliga R, Ueda N, Walker PD, Shah SV. Oxidant mechanisms in toxic acute renal failure. *Am J Kid Dis.* 1997;29:465–477.
56. Buttke TM, Sandstrom PA. Oxidative stress as a mediator of apoptosis. *Immunol Today.* 1994;15:7–10.
57. Jabs T. Reactive oxygen intermediates as mediators of programmed cell death in plants and animals. *Biochem Pharmacol.* 1999;57:231–245.
58. Takeda M, Shirato I, Kobayashi M, Endou H. Hydrogen peroxide induces necrosis, apoptosis, oncosis and apoptotic oncosis of mouse terminal proximal straight tubule cells. *Nephron.* 1999;81:234–238.
59. Kawabata T, Ma Y, Yamador I, Okada S. Iron-induced apoptosis in mouse renal proximal tubules after an injection of a renal carcinogen, iron-nitrilotriacetate. *Carcinogenesis.* 1997;18:1389–1394.
60. Duncan-Achanzar KB, Jones JT, Burke MF, et al. Inorganic mercury chloride-induced apoptosis in the cultured procine renal cell line LLC-PK1. *J Pharmacol Exp Therap.* 1996;277:1726–1732.
61. Nath KA, Grande JP, Croatt AJ, et al. Intracellular targets in heme protein-induced renal injury. *Kidney Int.* 1998;53:100–111.
62. Bauer MKA, Vogt M, Los M, et al. Role of reactive oxygen intermediates in activation-induced CD95 (Apo-1/Fas) ligand expression. *J Biol Chem.* 1998;273:8048–8055.
63. Uberti D, Yavin E, Gil S, et al. Hydrogen peroxide induces nuclear translocation of p53 and apoptosis in cells of oligodendroglia origin. *Mol Brain Res.* 1999;65:167–175.
64. Higuchi M, Honda T, Proske RJ, Yeh ETH. Regulation of reactive oxygen species-induced apoptosis and necrosis by caspase 3-like proteases. *Oncogene.* 1998;17:2753–2760.
65. Zager RA, Conrad DS, Burkhart K. Ceramide accumulation during oxidant renal tubular injury: mechanisms and potential consequences. *J Am Soc Nephrol.* 1998;9:1670–1680.
66. Ueda N, Hong X, Shah SV. Role of ceramide synthase in oxidant-induced ceramide generation in LLC-PK1 cells. *J Am Soc Nephrol.* 1998;9:417A.
67. Tournier C, Thomas G, Pierre J, et al. Mediation by arachidonic acid metabolites of the $H_2O_2$-induced stimulation of mitogen-activated protein kinases (extracellular-signal-regulated kinase and c-Jun $NH_2$-terminal kinase). *Eur J Biochem.* 1997;244:587–595.
68. Palmer HJ, Paulson KE. Reactive oxygen species and antioxidants in signal transduction and gene expression. *Nutr Rev.* 1997;55:353–361.
69. McGowan AJ, Ruiz-Ruiz MC, Gorman AM, et al. Reactive oxygen intermediate(s) (ROI): common mediator(s) of poly(ADP-ribose)polymerase (PARP) cleavage and apoptosis. *FEBS Lett.* 1996;392:299–303.
70. Xia Z, Dickens M, Raingeaud J, et al. Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis. *Science.* 1995;270:1326–1331.
71. Alnemri ES, Livingston DJ, Nicholson DW, et al. Human ICE/CED-3 protease nomenclature. *Cell.* 1996;87:171.
72. Yuan J, Shaham S, Ledoux S, et al. The C. elegans cell death gene ced-3 encodes a protein similar to mammalian interleukin-1b-converting enzyme. *Cell.* 1993;75:641–652.
73. Thornberry NA, Lazebnik Y. Caspases. Enemies within. *Science.* 1998;281:1312–1316.
74. Ahmad M, Srinivasula SM, Hegde R, et al. Identification and characterization of murine caspase14, a new member of the caspase family. *Cancer Res.* 1998;58:5201–5205.

75. Cohen GM. Caspases: the executioners of apoptosis. *Biochem J.* 1997;326:1–16.
76. Nicholson DW, Thornberry NA. Caspases: killer proteases. *Trends Biochem Sci.* 1997;22:299–306.
77. Liu X, Zou H, Slaughter C, Wang X. DFF, a heterodimeric protein that functions downstream of caspase-3 to trigger DNA fragmentation during apoptosis. *Cell.* 1997;89:175–184.
78. Enari M, Sakahira H, Yokoyama H, et al. A caspase-activated DNase that degrades DNA during apoptosis, and its inhibitor ICAD. *Nature.* 1998;391:43–50.
79. Kaushal GP, Ueda N, Shah SV. Role of caspases (ICE/CED 3 proteases) in DNA damage and cell death in response to a mitochondrial inhibitor, antimycin A. *Kidney Int.* 1997;52:438–445.
80. Kaushal GP, Singh AB, Shah SV. Identification of caspase (ICE-like proteases) gene family in rat kidney and altered expression in ischemia/reperfusion injury. *Am J Physiol.* 1998;274:F587–F595.
81. Feldenberg LR, Thevananther S, Del Rio M, et al. Partial ATP depletion induces Fas-and caspase-mediated apoptosis in MDCK cells. *Am J Physiol.* 1999;276:F837–F846.
82. Hannun YA. Functions of ceramide in coordinating cellular responses to stress. *Science.* 1996;274:1855–1859.
83. Kolesnick RN, Kronke M. Regulation of ceramide production and apoptosis. *Ann Rev Physiol.* 1998;60:643–665.
84. Zager RA, Iwata M, Conrad DS, et al. Altered ceramide and sphingosine expression during the induction phase of ischemic acute renal failure. *Kidney Int.* 1997;52:60–70.
85. Zager RA, Conrad S, Lochhead K, et al. Altered sphingomyelinase and ceramide expression in the setting of ischemic and nephrotoxic acute renal failure. *Kidney Int.* 1998;53:573–582.
86. Ueda N, Hong X, Shah SV. Role of ceramide synthase pathway in hypoxia/reoxygenation injury. *J Am Soc Nephrol.* 1998;9:417A.
87. Ueda N, Kaushal GP, Hong X, Shah SV. Role of enhanced ceramide generation in DNA damage and cell death in chemical hypoxic injury to LLC-PK1 cells. *Kidney Int.* 1998;54:399–406.
88. Soler AP, Mullin JM, Knudsen KA, Marano CW. Tissue remodeling during tumor necrosis factor-induced apoptosis in LLC-PK1 renal epithelial cells. *Am J Physiol.* 1996;270:F869–F879.
89. Kiyokawa N, Taguchi T, Mori T, et al. Induction of apoptosis in normal human renal tubular epithelial cells by *Echerichia coli* Shiga toxins 1 and 2. *J Infect Dis.* 1998;178:178–184.
90. Karpman D, Hakansson A, Perez MTR, et al. Apoptosis of renal cortical cells in the hemolytic-uremic syndrome: in vivo and in vitro studies. *Infect Immun.* 1998;66:636–644.
91. Harel Y, Silva M, Giroir B, et al. A reporter transgene indicates renal-specific induction of tumor necrosis factor (TNF) by Shiga-like toxin. *J Clin Invest.* 1993;92:2110–2116.
92. Mitra D, Jaffe EA, Weksler B, et al. Thrombotic thrombocytopenic purpura and sporadic hemolytic-uremic syndrome plasmas induce apoptosis in restricted lineages of human microvascular endothelial cells. *Blood.* 1997;89:1224–1234.
93. Mitra D, Kim J, MacLow C, et al. Role of caspases 1 and 3 and Bcl-2-related molecules in endothelial cell apoptosis associated with thrombotic microangiopathies. *Am J Hematol.* 1998;59:279–287.
94. Woods JS, Ellis ME, Dieguez-Acuna FJ, Corral J. Activation of NF-kB in normal rat kidney epithelial (NRK52E) cells is mediated via a redox-insensitive, calcium-dependent pathway. *Toxicol Appl Pharmacol.* 1999;154:219–227.
95. Jarpe MB, Widmann C, Knall C, et al. Anti-apoptotic versus pro-apoptotic signal transduction: checkpoints and stop signs along the road to death. *Oncogene.* 1998;17:1475–1482.
96. Baeuerle PA. Nf-kappa B: ten years after. *Cell.* 1996;87:13–20.
97. Takeda M, Kobayashi M, Shirato I, et al. Cisplatin-induced apoptosis of immortalized mouse proximal tubule cells is mediated by interleukin-1b converting enzyme (ICE) family of proteases but inhibited by overexpression of bcl-2. *Arch Toxicol.* 1997;71:612–621.
98. Takeda M, Kobayashi M, Shirato I, Endo H. Involvement of macromolecule synthesis, endonuclease activation and c-fos expression in cisplatin-induced apoptosis of mouse proximal tubule cells. *Toxicol Lett.* 1998;94:83–92.
99. Liu Y, Sun AM, Dworkin LD. Hepatocyte growth factor protects renal epithelial cells from apoptotic cell death. *Biochem Biophys Res Commun.* 1998;246:821–826.
100. Megyesi J, Safirstein RL, Price PM. Induction of p21WAF1/CIP1/SDI1 in kidney tubule cells affects the course of cisplatin-induced acute renal failure. *J Clin Invest.* 1998;101:777–782.
101. Herman EH, Zhang J, Hasinoff BB, et al. Comparison of the structural changes induced by doxorubicin and mitoxantrone in the heart, kidney and intestine and characterization of the Fe(III)-mitoxantrone complex. *J Mol Cell Cardiol.* 1997;29:2415–2430.
102. Thomas SE, Andoh TF, Pichler RH, et al. Accelerated apoptosis characterizes cyclosporine-associated interstitial fibrosis. *Kidney Int.* 1998;53:897–908.
103. Hizoh I, Strater J, Schick CS, et al. Radiocontrast-induced DNA fragmentation of renal tubular cells in vitro: role of hypertonicity. *Nephrol Dial Transplant.* 1998;13:911–918.
104. Loffing J, Loffing-Cueni D, Hegyi I, et al. Thiazide treatment of rats provokes apoptosis in distal tubule cells. *Kidney Int.* 1996;50:1180–1190.
105. Nouwen EJ, Verstrepen WA, Buyssens N, et al. Hyperplasia, hypertrophy, and phenotypic alterations in the distal nephron after acute proximal tubular injury in the rat. *Lab Invest.* 1994;70:479–493.
106. Dharnidharka VR, Nadeau K, Cannon CL, et al. Ciprofloxacin overdose: acute renal failure with prominent apoptotic changes. *Am J Kid Dis.* 1998;31:710–712.
107. Yan H, Carter CE, Xu C, et al. Cadmium-induced apoptosis in the urogenital organs of the male rat and its suppression by chelation. *J Toxicol Environ Health.* 1997;52:149–168.
108. Ishido M, Tohyama C, Suzuki T. C-myc is not involved in cadmium-elicited apoptotic pathway in porcine kidney LLC-PK1 cells. *Life Sci.* 1998;63:1195–1204.
109. Lieberthal W, Triaca V, Levine J. Mechanisms of death induced by cisplatin in proximal tubular epithelial cells: apoptosis versus necrosis. *Am J Physiol.* 1996;270:F700–F708.
110. Kawaida K, Matsumoto K, Shimazu H, Nakamura T. Hepatocyte growth factor prevents acute renal failure and accerelates renal regeneration in mice. *Proc Natl Acad Sci USA.* 1994;91:4357–4361.
111. Zhan Y, Cleveland JL, Stevens JL. A role of c-myc in chemically induced renal-cell death. *Mol Cell Biol.* 1997;17:6755–6764.
112. Fukuoka K, Takeda M, Kobayashi M, et al. Distinct interleukin-1b-converting enzyme family proteases mediate cisplatin- and staurosporine-induced apoptosis of mouse proximal tubule cells. *Life Sci.* 1998;62:1125–1138.
113. Van de Water B, Nagelkerke JF, Stevens JL. Dephosphorylation of focal adhesion kinase (FAK) and loss of focal contacts precede caspase-mediated cleavage of FAK during apoptosis in renal epithelial cells. *J Biol Chem.* 1999;274:13328–13337.
114. Ito H, Kasagi N, Shomori K, et al. Apoptosis in the human allografted kidney: analysis by terminal deoxynucleotidyl transferase-mediated DUTP-botin nick end labeling. *Transplantation.* 1995;60:794–798.
115. Wever PC, Aten J, Rentenaar RJ, et al. Apoptotic tubular cell death during acute renal allograft rejection. *Clin Nephrol.* 1998;49:28–34.
116. Laine J, Etelamaki P, Holmberg C, Dunkel L. Apoptotic cell death in human chronic renal allograft rejection. *Transplantation.* 1997;63:101–105.
117. Burns AT, Davies DR, McLaren AJ, et al. Apoptosis in ischemia/

reperfusion injury of human renal allografts. *Transplantation.* 1998;66:872–876.
118. Wang J, Nonomura N, Ichimaru N, et al. Expression of Fas and Fas ligand in renal grafts with acute and chronic rejection in the rat model. *J Interf Cyt Res.* 1997;17:369–373.
119. Kato S, Akasaka Y, Kawamura S. Fas antigen expression and its relationship with apoptosis in transplanted kidney. *Pathol Int.* 1997;47:230–237.
120. de Lajarte AS, Brun P, Baudouin V, et al. Detection of TIA-1 cytotoxic T-lymphocytes and epithelial apoptotic cells in renal allograft biopsies. *Transplant Proc.* 1995;27:2474–2475.
121. Nakopoulou L, Stefanaki K, Papadakis J, et al. Expression of bcl-2 oncoprotein in various types of glomerulonephritis and renal allografts. *Nephrol Dial Transplant.* 1996;11:997–1002.
122. Cummings MC. Increased p53 mRNA expression in liver and kidney apoptosis. *Biochim Biophys Acta.* 1996;1315:100–104.
123. Kennedy WA II, Buttyan R, Garcia-Montes E, et al. Epidermal growth factor suppresses renal tubular apoptosis following ureteral obstruction. *Urology.* 1997;49:973–980.
124. Chevalier RL, Goyal S, Wolstenholme JT, Thornhill BA. Obstructive nephropathy in the neonatal rat is attenuated by epidermal growth factor. *Kidney Int.* 1998;54:38–47.
125. Truong LD, Petrusevska G, Yang G, et al. Cell apoptosis and proliferation in experimental chronic obstructive uropathy. *Kidney Int.* 1996;50:200–207.
126. Morrissey JJ, Klahr S. Effect of AT2 receptor blockade on the pathogenesis of renal fibrosis. *Am J Physiol.* 1999;276:F39–F45.
127. Takemura T, Kondo S, Homma T, et al. The membrane-bound form of heparin-binding epidermal growth factor-like growth factor promotes survival of cultured renal epithelial cells. *J Biol Chem.* 1997;272:31036–31042.
128. Gobe GC, Buttyan R, Wyburn KRL, et al. Clusterin expression and apoptosis in tissue remodeling associated with renal regeneration. *Kidney Int.* 1995;47:411–420.